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BIOASSAY OF 2,5-DITHIOBIUREA FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

2,5-DITHIOBIUREA

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

REPORT ON BIOASSAY OF 2,5-DITHIOBIUREA FOR POSSIBLE CARCINOGENICITY

Availability

2,5-Dithiobiurea (CAS 142-46-1) has been tested for cancer-causing activity with rats and mice in the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute. A report is available to the public.

Summary: A bioassay of 2,5-dithiobiurea for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. Applications of the chemical include use as a component of photographic chemicals. 2,5-Dithiobiurea was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of high dose male rats, of which there were only 49.

Under the conditions of this bioassay, the evidence suggested, but was insufficient to establish the carcinogenicity of 2,5-dithiobiurea for female B6C3F1 mice. The compound was not carcinogenic to male B6C3F1 mice or to male or female Fischer 344 rats.

Single copies of the report, Bioassay of 2,5-Dithiobiurea for Possible Carcinogenicity (T.R. 132), are available from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

Dated: January 26, 1979

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Director
National Institutes of Health

(Catalogue of Federal Domestic Assistance Program Number 13.393, Cancer Cause and Prevention Research)

REPORT ON THE BIOASSAY OF 2,5-DITHIOBIUREA
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 2,5-dithiobiurea conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 2,5-dithiobiurea was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. R. W. Fleischman (3), Dr. A. S. Krishna Murthy (3), and Dr. D. S. Wyand (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5,8), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5,10), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,10), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,11), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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1. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

2. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.

3. Mason Research Institute, 57 Union Street, Worcester, Massachusetts.

4. Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri.

5. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

6. Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

7. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

8. Now with the Solar Energy Research Institute, Cole Boulevard, Golden, Colorado.

9. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

10. Now with Clement Associates, Inc., 1010 Wisconsin Avenue, N.W., Washington, D.C.

11. Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of 2,5-dithiobiurea for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. 2,5-Dithiobiurea was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of high dose male rats, of which there were only 49. The dietary concentrations used in the chronic bioassay were 0.6 percent for the low dose rats and 1.2 percent for the high dose rats. The dietary concentrations used for low and high dose mice were 1.0 and 2.0 percent, respectively. After a 78-week dosing period, observation of the rats continued for an additional 31 weeks and observation of the mice continued for an additional 16 weeks. For each species, 50 animals of each sex were placed on test as controls.

In both species, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was observed in mice but not in rats. No consistent pattern of clinical signs was observed in either species.

No tumors occurred at a significantly higher incidence in dosed rats than in their controls.

Among female mice, the Cochran-Armitage test indicated a significant positive association between the incidence of hepatocellular carcinoma and dietary concentration of 2,5-dithiobiurea. According to results of the Fisher exact test, the incidence of hepatocellular carcinoma was significantly higher in the high dose female mouse group when compared to the corresponding control group but not when compared to the laboratory historical control data. No neoplasms occurred at a significantly higher incidence in dosed male mice than in their controls.

Under the conditions of this bioassay, the evidence suggested, but was insufficient to establish the carcinogenicity of 2,5-dithiobiurea for female B6C3F1 mice. The compound was not carcinogenic to male B6C3F1 mice or to male or female Fischer 344 rats.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

2,5-Dithiobiurea (Figure 1) (NCI No. C03009), a component of photographic chemicals, was selected for bioassay by the National Cancer Institute because it is a dimer of thiourea, a liver, thyroid and Zymbal's gland tumorigen in rats (International Agency for Research on Cancer, 1974).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 1,2-hydrazinedicarbothioamide.*

2,5-Dithiobiurea can be used in both photographic emulsions (Kodak-Pathe, 1966; McBride, 1966) and bleach-fixing baths for color films (Nimura et al., 1973) and papers (Nimura et al., 1974). It can also be used as a fuel in pyrotechnic disseminating compositions (Niles, 1975), and electroplating baths for copper (Fujino and Fueki, 1971) and tin-nickel plating (Fueki at al., 1974).

Specific production data for 2,5-dithiobiurea are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value, annually) by one U.S. company (Stanford Research Institute, 1977).

The potential for exposure to 2,5-dithiobiurea is greatest for persons using photographic chemicals, pyrotechnic devices, and electroplating baths which contain this compound.

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*The CAS registry number is 142-46-1.

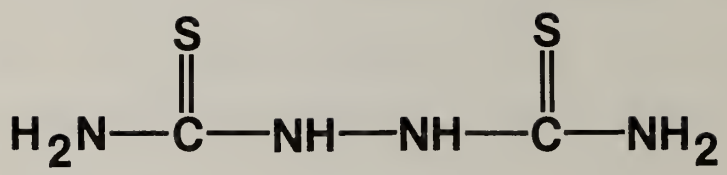


FIGURE 1
CHEMICAL STRUCTURE OF 2,5-DITHIOBIUREA

II. MATERIALS AND METHODS

A. Chemicals

Two batches of 2,5-dithiobiurea were purchased from Eastman Kodak Company, Rochester, New York by the NCI for Mason Research Institute, Worcester, Massachusetts. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri.

For the batch used during the first five months of the bioassay, the experimentally determined melting point range (205° to 208°C), although narrow, suggested the presence of at least minor impurities because of its deviation from the literature value of 214° to 215°C (Boit, 1973). Slight deviation of the experimentally determined elemental composition from $C_2H_6N_4S_2$, the molecular formula for 2,5-dithiobiurea, also indicated the presence of impurities. Thin-layer chromatography utilizing two solvent systems (ethyl acetate: methanol and acetone), each visualized with ultraviolet light, potassium dichromate, and heat, indicated the presence of one nonmotile impurity. High pressure liquid chromatography showed the presence of two impurities. Titration of the thiocarbonyl function provided a result that was approximately 94 percent of the theoretical value. This indicates that purity cannot exceed 94 percent, but other compounds containing thiocarbonyl functional groups could be present. Infrared analysis was consistent with the structure of the compound.

A second batch of the chemical, purchased five months later and used for the duration of the bioassay, appeared to be of lesser

purity since the range of the experimentally determined melting point for this batch (180° to 215°C) was wider. Results of elemental analysis approximated those expected for the molecular formula of the compound. Thin-layer chromatography utilizing two solvent systems (ethyl acetate:methanol and acetone), each visualized with 254 and 367 nm light, dichromate, and heat, indicated one nonmotile impurity. High pressure liquid chromatography also showed the presence of one impurity. Titration of the thiocarbonyl function provided a result that was 108 percent of the theoretical. The possible presence of impurities was supported by the results of infrared analysis and nuclear magnetic resonance analysis.

Throughout this report the term 2,5-dithiobiurea is used to represent these two batches of the chemical.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). 2,5-Dithiobiurea was administered to the dosed animals as a component of the diet. The chemical was mixed with an aliquot of feed. Once visual homogeneity was attained, the mixture was placed into a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender with the remainder of the meal. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixtures were discarded 2 weeks after formulation.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All animals used in the chronic bioassay were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, and all but the control mice were received in the same shipment. Control mice were received approximately 5 weeks after the other animals.

Upon arrival a sample of animals was examined for parasites and other signs of disease. The remaining animals were quarantined by species for 2 weeks prior to initiation of the test. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 16 months of the bioassay, rats were housed in galvanized-steel wire-mesh cages suspended above newspapers. Newspapers under

cages were replaced daily and cages and racks were washed weekly. For the remainder of the study, rats were maintained in suspended polycarbonate cages equipped with disposal nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. SAN-I-CEL® corncob bedding (Paxton Processing Company, Paxton, Illinois) was used during the first 7 months that the rats were housed in polycarbonate cages, while Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was used for the remainder of the bioassay. Stainless steel cage racks were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate shoe box type cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless steel lids. Stainless steel wire bar lids were used during the final observation period. Both types of lids were from Lab Products, Inc., Garfield, New Jersey. Nonwoven fiber filter bonnets were used over cage lids. Dosed mice were housed ten per cage for the first 15 months of study and five per cage thereafter. Control mice, initially housed ten per cage, were changed to five per cage after 13 months. Clean cages, lids, and bedding were provided three times per week when cage populations were reduced to five. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used for the first 3 months of the bioassay (only 2 months for controls). SAN-I-CEL® was used as bedding for the next 12 months, after which a second corncob bedding

(Bed-o-Cobs®, The Andersons Cob Division, Maumee, Ohio) was provided for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Tap water was available ad libitum for both species from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, refilled as needed between changes.

Wayne Lab-Blox® was supplied ad libitum throughout the bioassay. Animals received Wayne Lab-Blox® meal during the initial quarantine and periods of compound administration. Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) containing stainless steel baffles were used to distribute powdered feed for the first 13 months of study for all rats, for the first 17 months for dosed mice and for the first 16 months for control mice. For the remainder of the period of compound administration, meal was available from stainless steel gangstyle feed hoppers (Scientific Cages, Inc., Bryan, Texas). During the final observation period, mice were fed pellets from a wire bar hopper incorporated into the cage lid and rats were fed pellets on the cage floor. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

2,5-Dithiobiurea-dosed and control rats were housed in a room with rats intubated with* m-cresidine (102-50-1); and with other rats

*CAS registry numbers are given in parentheses.

receiving diets containing fenaminosulf (140-56-7) and cupferron (135-20-6).

All mice, including controls, in the 2,5-dithiobiurea study were housed in a room with other mice receiving diets containing fenaminosulf (140-56-7); cupferron (135-20-6); 4-chloro-o-phenylenediamine (95-83-0); o-anisidine hydrochloride (134-29-0); and p-anisidine hydrochloride (20265-97-8).

E. Selection of Initial Concentrations

In order to establish the concentrations of 2,5-dithiobiurea for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among several groups (five for rats and six for mice), each consisting of five males and five females. 2,5-Dithiobiurea was incorporated into the basal laboratory diet and supplied ad libitum to four of the five rat groups in concentrations of 0.3, 0.15, 0.08, and 0.04 percent and five of the six mouse groups in concentrations of 2.0, 1.0, 0.5, 0.25, and 0.125 percent. The fifth rat group and the sixth mouse group served as control groups, receiving only the basal diet. The dosed dietary preparations were administered for a period of 7 weeks, followed by a 1-week observation period during which all animals were fed the basal laboratory diet.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean group body weight depression in

excess of 20 percent relative to controls was selected as the high concentration utilized for the chronic bioassay.

In rats, no deaths were observed and no gross pathology was recorded at necropsy. Mean group body weight depression was 8.9 and 9.2 percent, respectively, in males and females receiving 0.3 percent 2,5-dithiobiurea, the highest concentration administered. The concentration selected for high dose male and female rats in the chronic study was 1.2 percent.

For mice, no deaths were observed and no gross pathology was recorded at necropsy. Mean group body weight depression was 24.0 and 11.7 percent, respectively, in males and females receiving 1.0 percent, while it was 16.0 and 15.5 percent, respectively, in males and females receiving 2.0 percent 2,5-dithiobiurea. The concentration selected for high dose male and female mice in the chronic study was 2.0 percent.

F. Experimental Design

The experimental design parameters for the chronic bioassay (species, sex, group size, actual concentrations administered and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

At initiation of the bioassay all rats were approximately 6 weeks old and shared the same median date of birth. The doses of 2,5-dithiobiurea utilized for both male and female rats were 1.2 and 0.6 percent. Throughout this report those rats receiving the former

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2,5-DITHIOBIUREA FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,5-DITHIOBIUREA CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	110
LOW DOSE	50	0.6 0	78	31
HIGH DOSE	49	1.2 0	78	31
FEMALE				
CONTROL	50	0	0	110
LOW DOSE	50	0.6 0	78	31
HIGH DOSE	50	1.2 0	78	31

[a]Concentrations given in percentages in feed.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2,5-DITHIOBIUREA FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,5-DITHIOBIUREA CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	98
LOW DOSE	50	1.0	78	
		0		16
HIGH DOSE	50	2.0	78	
		0		16
FEMALE				
CONTROL	50	0	0	98
LOW DOSE	50	1.0	78	
		0		16
HIGH DOSE	50	2.0	78	
		0		16

[a]Concentrations given in percentages in feed.

concentration are referred to as the high dose groups, while those receiving the latter concentration are referred to as the low dose groups. These concentrations were administered in the feed for a period of 78 weeks, followed by an observation period of up to 31 weeks. Control rats were on test for 110 weeks.

At initiation of the bioassay all dosed mice were approximately 6 weeks old and shared the same median date of birth. Control mice were approximately 7 weeks old when they were started on test approximately 5 weeks after the dosed mice. Control mice were observed for 98 weeks. The doses utilized for both male and female mice were 2.0 and 1.0 percent. Throughout this report those mice receiving the former concentration are referred to as the high dose groups, while those receiving the latter concentration are refered to as the low dose groups. These concentrations were administered in the feed for 78 weeks, followed by an observation period of up to 16 weeks.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. From the first day, all animals were inspected twice daily. The presence of

tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was killed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. Gross and microscopic examinations were performed on all major tissues, organs and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined

microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when

testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was

used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week

during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group

would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

No compound-related mean body weight depression was apparent in dosed male or female rats when compared to controls (Figure 2).

Only isolated clinical signs were observed. Subcutaneous masses developed on the hind leg in two high dose males and in the axillary mammary region in one low dose male and one high dose female. One low dose male developed a cutaneous lesion of the chin and one control male had a hard cutaneous lesion on the dorsal surface.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 2,5-dithiobiurea-dosed groups are shown in Figure 3. The Tarone test for positive association between dosage and mortality was significant for both males and females.

For males five rats from the high dose and five from the control group were sacrificed in week 78. Survival was relatively high in all groups until about week 70, after which increased mortality was seen--especially in the high dose group. Adequate numbers of male rats were at risk from late-developing tumors, with 22/50 (44 percent) of the high dose, 38/50 (76 percent) of the low dose, and 32/50 (64 percent) of the control rats surviving on test until the termination of the study.

For females five rats from the high dose and five from the control group were sacrificed in week 78. However, survival was also

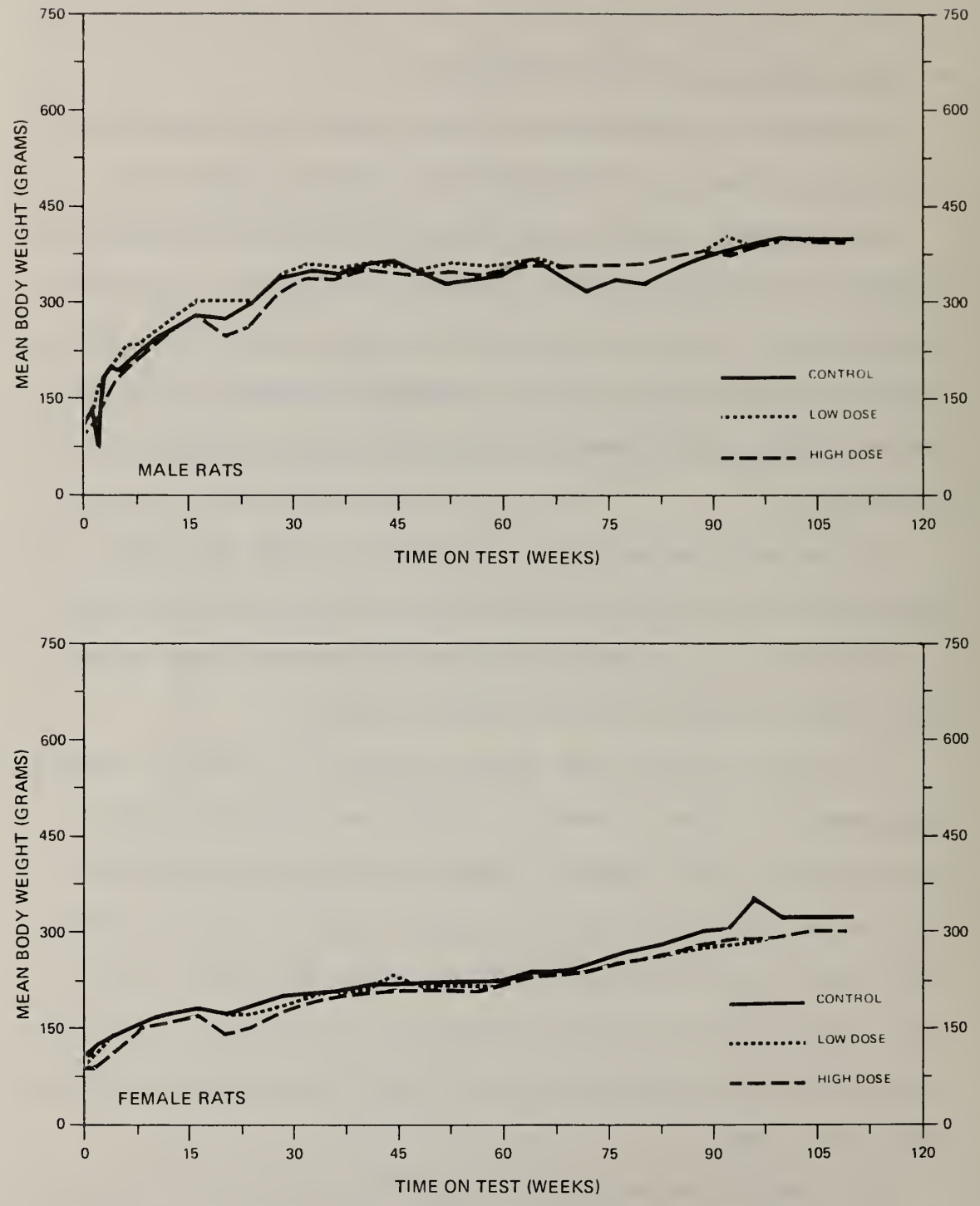
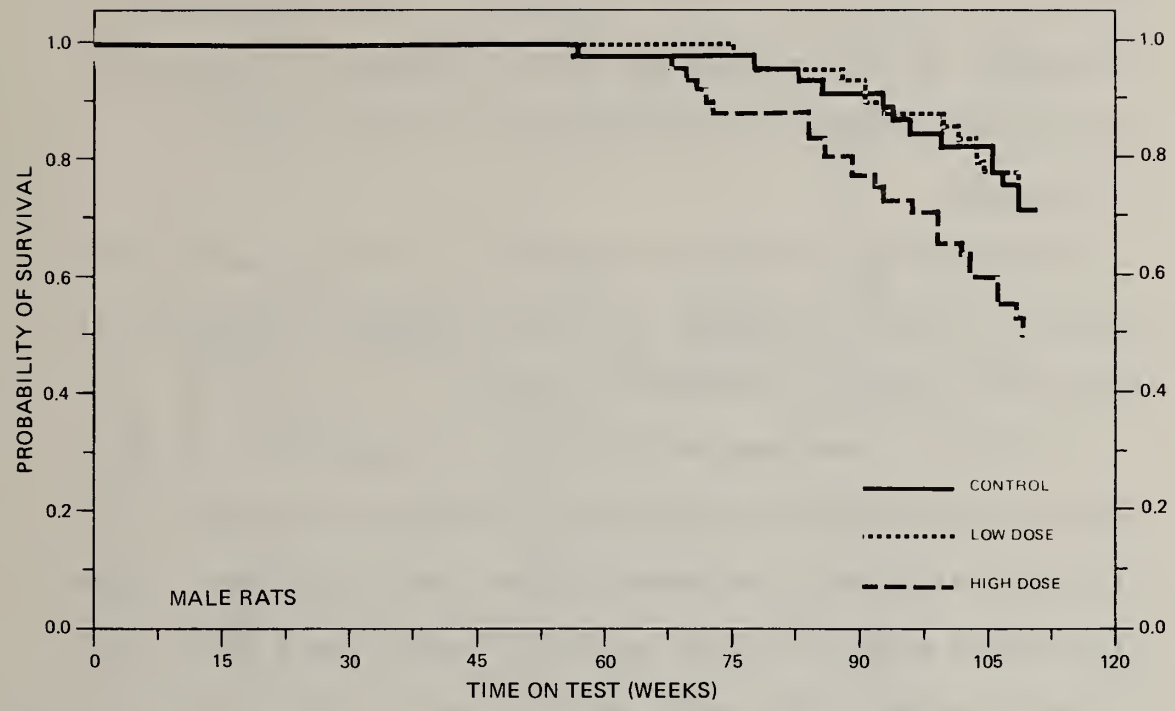


FIGURE 2
GROWTH CURVES FOR 2,5-DITHIOBIUREA CHRONIC STUDY RATS



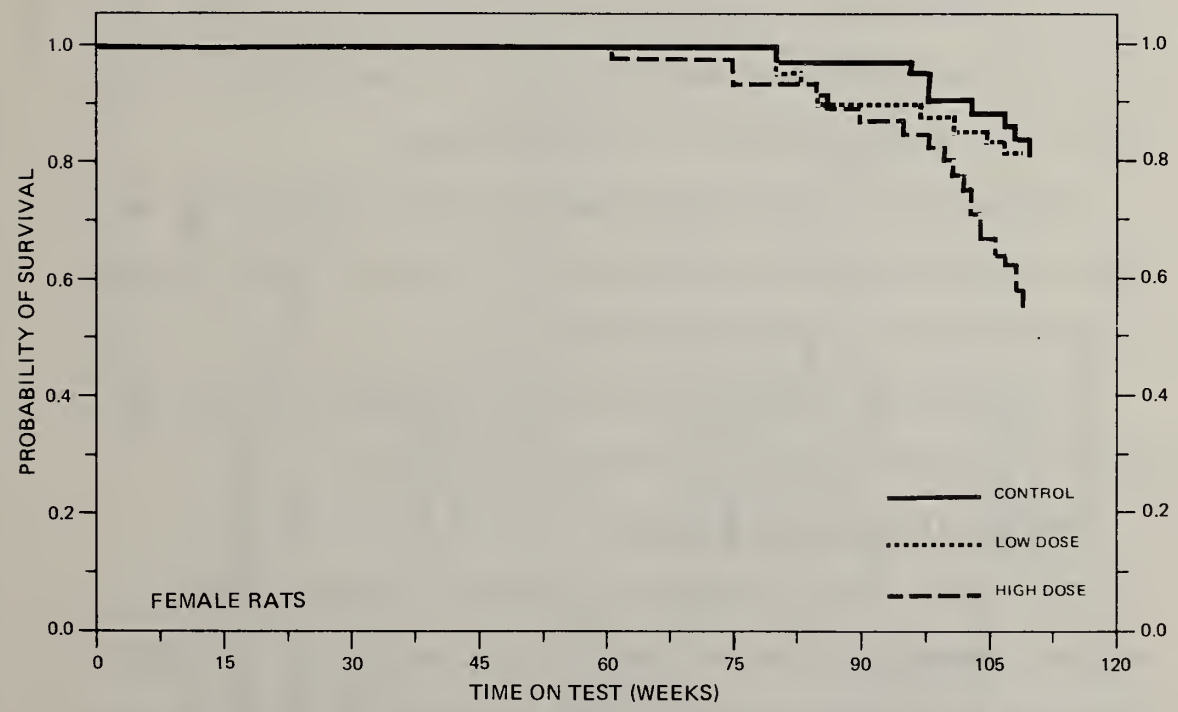


FIGURE 3
SURVIVAL COMPARISONS OF 2,5-DITHIOBIUREA CHRONIC STUDY RATS

adequate for females, with 24/50 (48 percent) of the high dose, 41/50 (82 percent) of the low dose, and 36/50 (72 percent) of the control rats surviving on test until the termination of the study.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

A variety of neoplasms was observed with approximately equal frequency in the dosed and control rats. There were instances in this study, as noted in the summary tables, where neoplasms occurred only in dosed animals, or with increased frequency when compared to the control animals. The nature and incidence of these lesions were similar to those known to occur spontaneously in aged Fischer 344 rats, and therefore, these neoplasms were not considered to be related to the administration of 2,5-dithiobiurea.

Nonneoplastic lesions which commonly occur in aging rats of this strain were seen in dosed and control rats. None of these lesions was considered to be compound-induced.

This pathology examination provided no evidence for the carcinogenicity of 2,5-dithiobiurea in Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2,5-DITHIOBIUREA[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia[b]	10/50(0.20)	10/49(0.20)	14/48(0.29)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.020	1.458
Lower Limit	---	0.419	0.670
Upper Limit	---	2.484	3.298
Weeks to First Observed Tumor	78	78	73
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	10/50(0.20)	11/49(0.22)	14/48(0.29)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.122	1.458
Lower Limit	---	0.477	0.670
Upper Limit	---	2.674	3.298
Weeks to First Observed Tumor	78	78	73
Pituitary: Carcinoma NOS[b]	0/45(0.00)	0/44(0.00)	4/39(0.10)
P Values[c]	P = 0.011	N.S.	P = 0.043
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	1.075
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	109

Table 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS or Carcinoma NOS or Chromophobe Adenoma[b]	7/45(0.16)	6/44(0.14)	6/39(0.15)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.877	0.989
Lower Limit	---	0.264	0.299
Upper Limit	---	2.801	3.134
Weeks to First Observed Tumor	78	104	95
Adrenal: Pheochromocytoma[b]	3/50(0.06)	4/49(0.08)	7/46(0.15)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.361	2.536
Lower Limit	---	0.243	0.619
Upper Limit	---	8.854	14.390
Weeks to First Observed Tumor	78	109	106
Thyroid: Follicular-Cell Carcinoma[b]	1/37(0.03)	3/41(0.07)	2/37(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.707	2.000
Lower Limit	---	0.230	0.109
Upper Limit	---	138.498	114.740
Weeks to First Observed Tumor	110	109	109

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	2/37(0.05)	2/41(0.05)	1/37(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.902	0.500
Lower Limit	---	0.069	0.009
Upper Limit	---	11.920	9.179
Weeks to First Observed Tumor	109	109	109
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/37(0.08)	2/41(0.05)	2/37(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.602	0.667
Lower Limit	---	0.053	0.058
Upper Limit	---	4.969	5.481
Weeks to First Observed Tumor	109	109	109
Testis: Interstitial-Cell Tumor[b]	42/50(0.84)	47/48(0.98)	33/47(0.70)
P Values[c]	P = 0.049(N)	P = 0.018	N.S.
Departure from Linear Trend[e]	P = 0.002	---	---
Relative Risk (Control)[d]	---	1.166	0.836
Lower Limit	---	1.009	0.675
Upper Limit	---	1.218	1.065
Weeks to First Observed Tumor	78	78	70

TABLE 3 (Concluded)

[a]Treated groups received doses of 0.6 or 1.2 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 2,5-DITHIOBIUREA[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia[b]	7/49(0.14)	7/50(0.14)	5/49(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.980	0.714
Lower Limit	---	0.317	0.191
Upper Limit	---	3.032	2.430
Weeks to First Observed Tumor	96	101	90
Pituitary: Carcinoma NOS[b]	0/39(0.00)	3/41(0.07)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.577	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	109	---
Pituitary: Adenoma NOS or Chromophobe Adenoma or Carcinoma NOS[b]	17/39(0.44)	15/41(0.37)	12/45(0.27)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.839	0.612
Lower Limit	---	0.461	0.311
Upper Limit	---	1.524	1.183
Weeks to First Observed Tumor	78	97	78

TABLE 4 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	2/45(0.04)	5/46(0.11)	1/32(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.446	0.703
Lower Limit	---	0.425	0.012
Upper Limit	---	24.643	12.848
Weeks to First Observed Tumor	110	109	109
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	2/45(0.04)	6/46(0.13)	1/32(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.935	0.703
Lower Limit	---	0.560	0.012
Upper Limit	---	28.500	12.848
Weeks to First Observed Tumor	110	109	109
Mammary: Fibroadenoma[b]	12/49(0.24)	6/50(0.12)	9/49(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.490	0.750
Lower Limit	---	0.164	0.308
Upper Limit	---	1.291	1.757
Weeks to First Observed Tumor	103	80	75

TABLE 4 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	5/46(0.11)	9/49(0.18)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.690	0.800
Lower Limit	---	0.553	0.169
Upper Limit	---	5.969	3.480
Weeks to First Observed Tumor	110	109	75
Adrenal: Pheochromocytoma[b]	3/49(0.06)	0/48(0.00)	1/48(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.340
Lower Limit	---	0.000	0.007
Upper Limit	---	1.695	4.060
Weeks to First Observed Tumor	110	---	109

[a]Treated groups received doses of 0.6 or 1.2 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

tumors were observed in at least one of the control or 2,5-dithiobiurea-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats the Cochran-Armitage test for the incidence of pituitary carcinoma NOS was significant (P = 0.011). The Fisher exact test comparing the incidence of this tumor in the high dose group to that in the control group yielded a probability level of P = 0.043, a marginal result which was not significant under the Bonferroni criterion. When the combined incidences of pituitary carcinoma NOS, pituitary adenoma NOS, and pituitary chromophobe adenoma was considered, however, no tests were significant.

In male rats the Cochran-Armitage test showed a significant (P = 0.049) negative association between dose and the incidence of interstitial-cell tumors of the testis. The Fisher exact test, however, showed a significantly (P = 0.018) increased incidence of interstitial-cell tumors in the low dose group compared to the control. The comparison of high dose to control was not significant.

Based upon these results there was insufficient evidence to conclude that 2,5-dithiobiurea was a carcinogen in rats. No other statistical tests for any site in rats of either sex indicated a significant positive association between the administration of 2,5-dithiobiurea and tumor incidence.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative

risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2,5-dithiobiurea that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Compound-related mean body weight depression was apparent in both male and female mice from weeks 20 through 84 (Figure 4). There was no difference in mean body weight gain of low and high dose mice.

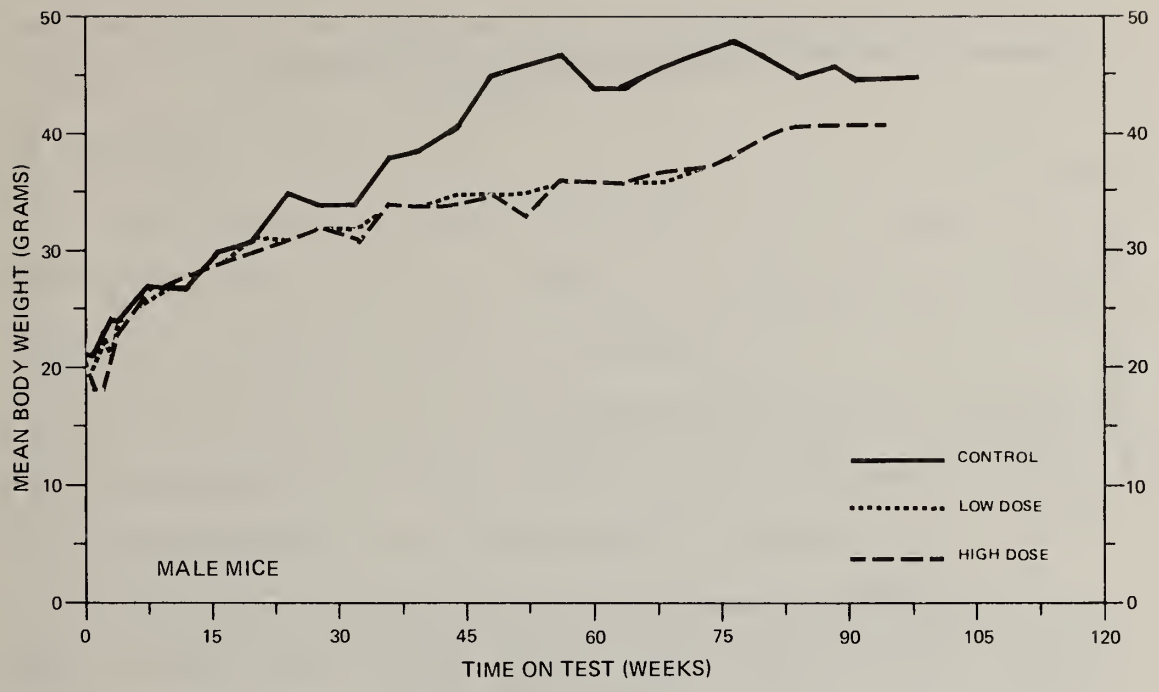
No unusual signs were recorded for mice of either sex.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 2,5-dithiobiurea-dosed groups are shown in Figure 5. For male mice the Tarone test for a positive association between dosage and mortality was significant. For female mice the Tarone test did not show a significant positive association between dosage and mortality.

For males five mice were sacrificed from the high dose group in week 78 and five from the control group in week 79. Adequate numbers of male mice were at risk from late-developing tumors, with 35/50 (70 percent) of the high dose, 49/50 (98 percent) of the low dose, and 42/50 (84 percent) of the control group surviving on test until the termination of the study.

For females five mice were sacrificed from the high dose group in week 78 and five from the control group in week 79. Survival among females was also adequate, with 40/50 (80 percent) of the high dose, 42/50 (84 percent) of the low dose, and 40/50 (80 percent) of



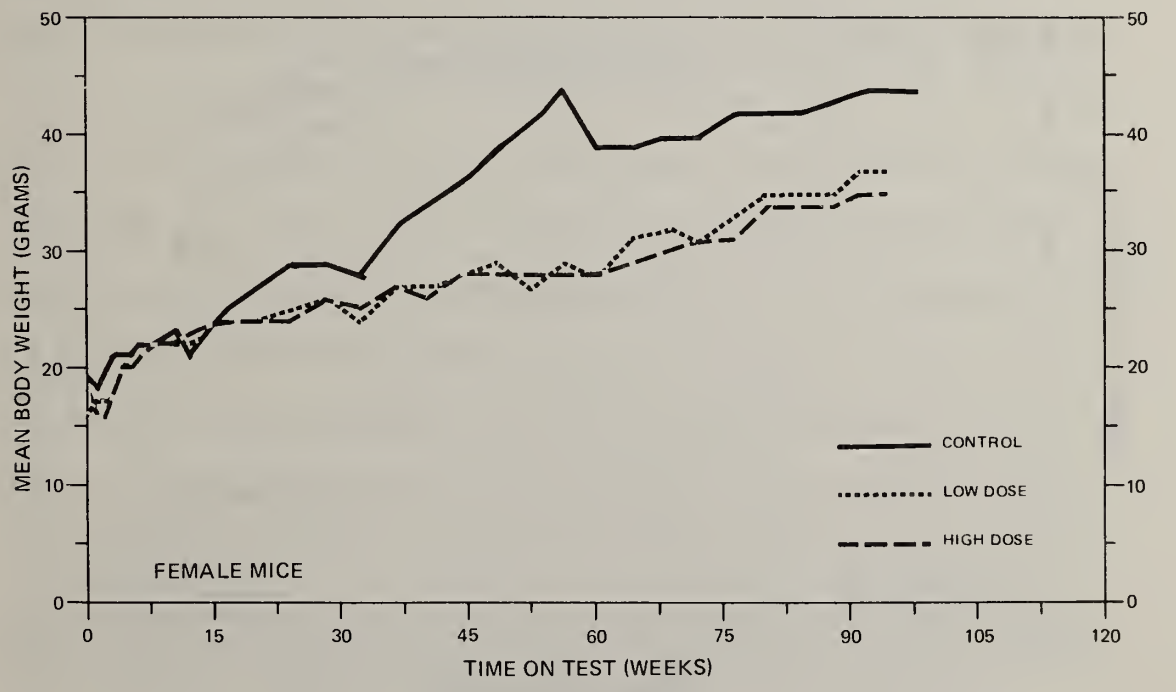


FIGURE 4
GROWTH CURVES FOR 2,5-DITHIOBIUREA CHRONIC STUDY MICE

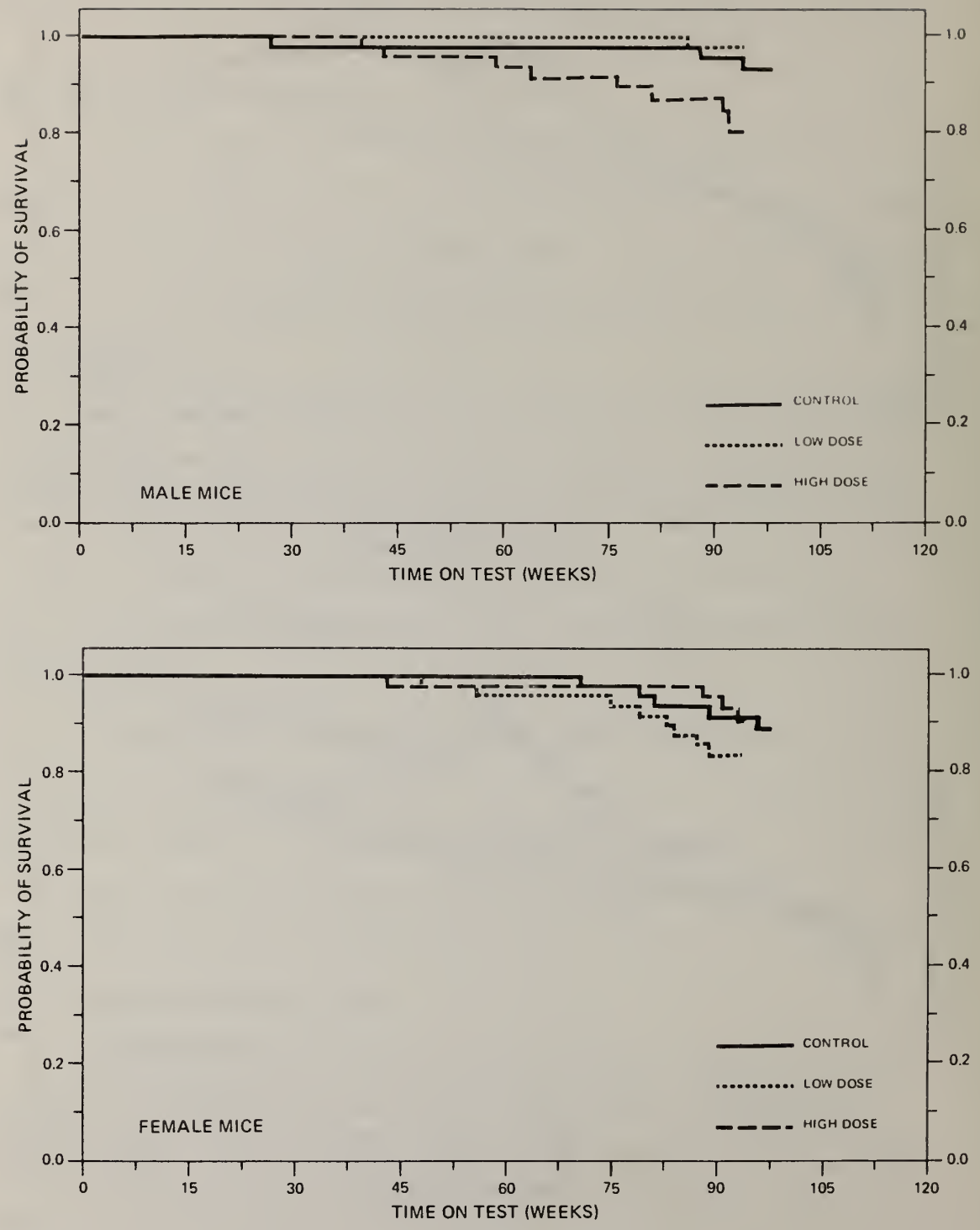


FIGURE 5
SURVIVAL COMPARISONS OF 2,5-DITHIOBIUREA CHRONIC STUDY MICE

the control group surviving on test until the termination of the study.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

With the exception of liver neoplasms observed in the female mice, the neoplasms observed in dosed mice were noted at incidences similar to those which occur spontaneously in B6C3F1 mice.

The incidence of hepatocellular carcinomas in both low (8/47 [17 percent]) and high dose (9/48 [19 percent]) female mice was elevated when compared with the control female mice (2/49 [4 percent]). In addition, hyperplastic nodules were found in a few dosed female mice (3/47 [6 percent] low dose and 2/48 [4 percent] high dose). Histologically, the hepatocellular carcinomas varied from well-differentiated neoplasms with rather close resemblance to normal liver to neoplasms with greater architectural and cytological deviation from normal liver. In the less well-differentiated neoplasms, there were cytoplasmic vacuolation, great variation in cell size, and cytoplasmic hyaline bodies. Well-differentiated neoplasms were composed of nests and cords of cells, and they lacked bile ducts. They compressed the normal parenchyma. Some contained focal areas of more undifferentiated cells. Undifferentiated tumors commonly had cystic and

blood-filled spaces. Cords and nests of atypical cells were often separated by dilated blood-filled sinusoids. A great variation in the incidence of mitotic figures was observed. Metastases did not occur in the dosed mice but did occur at a very low frequency in both male and female controls. No compound-related effects on the livers of male mice were observed.

A variety of inflammatory and degenerative lesions which commonly occur in mice of this strain was seen with approximately equal frequency in the dosed and control mice. These nonneoplastic lesions were not considered to be compound-induced.

Based upon this pathology examination, 2,5-dithiobiurea was carcinogenic to female mice. There was an increased incidence of hepatocellular carcinomas in dosed female mice when compared to control female mice. Compound-related neoplasms were not observed in the male mice in this study.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2,5-dithiobiurea-dosed groups and where such tumors were observed in at least 5 percent of the group.

For female mice the Cochran-Armitage test indicated a significant ($P = 0.023$) positive association between dose and the incidence

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 2,5-DITHIOBIUREA[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/47(0.06)	3/50(0.06)	2/47(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.940	0.667
Lower Limit	---	0.132	0.058
Upper Limit	---	6.700	5.554
Weeks to First Observed Tumor	98	94	94
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	7/47(0.15)	13/50(0.26)	4/47(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.746	0.571
Lower Limit	---	0.714	0.131
Upper Limit	---	4.722	2.089
Weeks to First Observed Tumor	97	94	94
Hematopoietic System: Malignant Lymphoma[b]	1/50(0.02)	4/50(0.08)	4/47(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	4.000	4.255
Lower Limit	---	0.415	0.442
Upper Limit	---	192.807	204.823
Weeks to First Observed Tumor	97	86	94

TABLE 5 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	15/49(0.31)	9/50(0.18)	7/47(0.15)
P Values[c]	P = 0.039(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.588	0.487
Lower Limit	---	0.252	0.185
Upper Limit	---	1.292	1.144
Weeks to First Observed Tumor	94	94	92

[a]Treated groups received doses of 1.0 or 2.0 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2,5-DITHIOBIUREA[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/50(0.06)	1/47(0.02)	1/48(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.355	0.347
Lower Limit	---	0.007	0.007
Upper Limit	---	4.229	4.143
Weeks to First Observed Tumor	79	83	94
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	4/50(0.08)	5/47(0.11)	6/48(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.330	1.563
Lower Limit	---	0.304	0.396
Upper Limit	---	6.316	7.090
Weeks to First Observed Tumor	79	83	94
Hematopoietic System: Malignant Lymphoma[b]	6/50(0.12)	12/48(0.25)	10/48(0.21)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.083	1.736
Lower Limit	---	0.792	0.622
Upper Limit	---	6.219	5.365
Weeks to First Observed Tumor	98	48	78

TABLE 6 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	2/49(0.04)	8/47(0.17)	9/48(0.19)
P Values[c]	P = 0.023	P = 0.039	P = 0.023
Relative Risk (Control)[d]	---	4.170	4.594
Lower Limit	---	0.889	1.017
Upper Limit	---	38.627	41.865
Weeks to First Observed Tumor	98	94	78
Pituitary: Adenoma NOS[b]	0/42(0.00)	1/44(0.02)	2/37(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.051	0.338
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	94	94

[a]Treated groups received doses of 1.0 or 2.0 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

of hepatocellular carcinoma. This was supported by a significant (P = 0.023) Fisher exact test comparing the incidence of this tumor in the high dose group to that in the control group. The low dose to control comparison had a probability level of P = 0.039, a marginal result which was not significant under the Bonferroni criterion. In historical data on untreated B6C3F1 mice at Mason Research Institute in the NCI Carcinogenesis Testing Program, 19/275 (7 percent) control female mice had this tumor--compared to the incidences in this bioassay of 2/49 (4 percent), 8/47 (17 percent), and 9/48 (19 percent) observed in the control, low dose, and high dose groups, respectively. This, together with the fact that the control mice were not matched, weakened the significance of the findings.

For male mice the Cochran-Armitage test for the incidence of hepatocellular carcinoma showed a significant (P = 0.039) negative association. The Fisher exact tests, however, were not significant. The historical incidence of this tumor in male B6C3F1 untreated control mice observed at Mason Research Institute was 88/275 (32 percent), compared to the incidence of 15/49 (31 percent) in the controls for this bioassay.

Based on these statistical results, the administration of 2,5-dithiobiurea was associated with an elevated incidence of hepatocellular carcinoma in female B6C3F1 mice under the conditions of this experiment. No other statistical tests for mice of either sex were significant.

V. DISCUSSION

In both species adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was observed in mice but not in rats. No consistent pattern of clinical signs was observed in either species.

In rats no tumors occurred at a significantly higher incidence in groups of rats dosed with 2,5-dithiobiurea than in corresponding control groups. Since no significant retardation of growth, or increased occurrence of clinical signs were associated with the feeding of 2,5-dithiobiurea, it is possible that the compound was not administered to rats at the maximum tolerated concentration.

Among female mice, there was a significant positive association between the incidence of hepatocellular carcinoma and the concentration of 2,5-dithiobiurea in the diet. The incidence of hepatocellular carcinoma was significantly higher in the high dose group than in the control group. The control group was not completely matched, however, since it was started 5 weeks after the dosed animals, and the control incidence of 4 percent hepatocellular carcinomas was lower than the 7 percent found in the laboratory's historical controls. Among male mice, however, there was a significant negative association between the incidence of hepatocellular carcinoma and dietary concentration. No neoplasms occurred at a significantly higher incidence in dosed male mice than in their controls.

Under the conditions of this bioassay, the evidence suggested that 2,5-dithiobiurea was carcinogenic to female B6C3F1 mice, causing an increased incidence of hepatocellular carcinomas, but was not carcinogenic to male B6C3F1 mice or to Fischer 344 rats of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2,5-DITHIOBIUREA

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 2,5-DITHIOBIUREA

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(48)
FIBROMA	1 (2%)		
FIBROSARCOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(49)	(48)
SARCOMA, NOS	1 (2%)		
FIBROMA	1 (2%)		1 (2%)
FIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(48)
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	9 (18%)	10 (20%)	11 (23%)
GRANULOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(50)	(49)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#LIVER	(49)	(49)	(48)
MYELOMONOCYTIC LEUKEMIA			2 (4%)
CIRCULATORY SYSTEM			
#HEART	(48)	(48)	(47)
SARCOMA, NOS, METASTATIC	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(48)
HEPATOCELLULAR CARCINOMA		1 (2%)	2 (4%)
#JEJUNUM	(49)	(48)	(45)
LEIOMYOSARCOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(44)	(39)
CARCINOMA,NOS			4 (10%)
ADENOMA, NOS	5 (11%)	6 (14%)	2 (5%)
CHROMOPHOBE ADENOMA	2 (4%)		
#ADRENAL	(50)	(49)	(46)
PHEOCHROMOCYTOMA	3 (6%)	4 (8%)	6 (13%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(37)	(41)	(37)
FOLLICULAR-CELL CARCINOMA	1 (3%)	3 (7%)	2 (5%)
C-CELL ADENOMA	1 (3%)	1 (2%)	1 (3%)
C-CELL CARCINOMA	2 (5%)	2 (5%)	1 (3%)
#PANCREATIC ISLETS	(47)	(48)	(45)
ISLET-CELL ADENOMA	1 (2%)	2 (4%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(49)	(48)
CARCINOMA,NOS	2 (4%)		1 (2%)
ADENOMA, NOS			1 (2%)
#TESTIS	(50)	(48)	(47)
INTERSTITIAL-CELL TUMOR	42 (84%)	47 (98%)	33 (70%)
NERVOUS SYSTEM			
#BRAIN	(50)	(48)	(46)
ASTROCYTOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
#CEREBRAL CORTEX	(50)	(48)	(46)
GLIOMA, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(49)	(48)
CERUMINOUS CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(50)	(49)	(48)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*BODY CAVITIES	(50)	(49)	(48)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
MESOTHELIOMA, MALIGNANT			1 (2%)
*ABDOMINAL CAVITY	(50)	(49)	(48)
LEIOMYOSARCOMA		1 (2%)	
ALL OTHER SYSTEMS			
THORACIC CAVITY			
HEPATOCELLULAR CARCINOMA, METAST			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	5	11
MORIBUND SACRIFICE	8	7	12
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32	38	22
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	48	40
TOTAL PRIMARY TUMORS	76	82	75
TOTAL ANIMALS WITH BENIGN TUMORS	46	47	35
TOTAL BENIGN TUMORS	56	62	45
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	18	24
TOTAL MALIGNANT TUMORS	20	19	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		1
TOTAL SECONDARY TUMORS	1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 2,5-DITHIOBIUREA

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
OSTEOSARCOMA			1 (2%)
*SUBCUT TISSUE	(49)	(50)	(49)
FIBROMA	2 (4%)	1 (2%)	
FIBROSARCOMA		1 (2%)	
LIPOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
*LUNG	(49)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(49)
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	6 (12%)	5 (10%)	5 (10%)
*MEDIASTINAL L.NODE	(44)	(46)	(41)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
*LIVER	(48)	(50)	(49)
MYELOMONOCYTIC LEUKEMIA		2 (4%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
DIGESTIVE SYSTEM			
#LIVER	(48)	(50)	(49)
NEOPLASTIC NODULE			1 (2%)
HEPATOCELLULAR CARCINOMA	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (2%)
TUBULAR-CELL ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(41)	(45)
CARCINOMA,NOS		3 (7%)	
ADENOMA, NOS	15 (38%)	12 (29%)	12 (27%)
CHROMOPHOBE ADENOMA	2 (5%)		
#ADRENAL	(49)	(48)	(48)
PHEOCHROMOCYTOMA	3 (6%)		
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#ADRENAL CORTEX	(49)	(48)	(48)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#THYROID	(45)	(46)	(32)
FOLLICULAR-CELL CARCINOMA			1 (3%)
C-CELL ADENOMA		1 (2%)	
C-CELL CARCINOMA	2 (4%)	5 (11%)	1 (3%)
#PARATHYROID	(27)	(35)	(18)
ADENOMA, NOS			1 (6%)
REPRODUCTIVE SYSTEM			
#MAMMARY GLAND	(49)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)
FIBROADENOMA	12 (24%)	6 (12%)	9 (18%)
#CLITORAL GLAND	(49)	(50)	(49)
CARCINOMA,NOS		2 (4%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
ADENOMA, NOS	1 (2%)		
#UTERUS	(46)	(49)	(46)
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP	5 (11%)	9 (18%)	4 (9%)
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(48)
ASTROCYTOMA	1 (2%)		
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(49)	(50)	(49)
CERUMINOUS CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKULL	(49)	(50)	(49)
OSTEOMA		1 (2%)	
BODY CAVITIES			
*PERITONEUM	(49)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
DIAPHRAGM			
ADENOCARCINOMA, NOS, METASTATIC			1
SITE UNKNOWN			
ADENOCARCINOMA, NOS			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	5	13
MORIBUND SACRIFICE	6	4	8
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	41	24
ANIMAL MISSING	1		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	36	32
TOTAL PRIMARY TUMORS	52	51	42
TOTAL ANIMALS WITH BENIGN TUMORS	27	25	21
TOTAL BENIGN TUMORS	40	32	27
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	16	14
TOTAL MALIGNANT TUMORS	12	19	14
TOTAL ANIMALS WITH SECONDARY TUMORS#			3
TOTAL SECONDARY TUMORS			7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 2,5-DITHIOBIUREA

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 2,5-DITHIOBIUREA

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	49	50	47
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(47)
SQUAMOUS CELL PAPILLOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(47)	(50)	(47)
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (9%)	10 (20%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	3 (6%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(47)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SPLEEN	(49)	(50)	(47)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA	1 (2%)	1 (2%)	1 (2%)
MALIGNANT LYMPHOMA, NOS			2 (4%)
#MEDIASTINAL L.NODE	(40)	(41)	(40)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#PANCREATIC L.NODE	(40)	(41)	(40)
MALIGNANT LYMPHOMA, NOS			1 (3%)
#DUODENUM	(49)	(50)	(46)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
DIGESTIVE SYSTEM			
*LIVER	(49)	(50)	(47)
HEPATOCELLULAR CARCINOMA	15 (31%)	9 (18%)	7 (15%)
*STOMACH	(49)	(50)	(47)
ADENOMATOUS POLYP, NOS	1 (2%)		
URINARY SYSTEM			
*KIDNEY	(49)	(50)	(47)
TUBULAR-CELL ADENOMA			1 (2%)
ENDOCRINE SYSTEM			
*THYROID	(42)	(47)	(40)
FOLLICULAR-CELL ADENOMA			1 (3%)
FOLLICULAR-CELL CARCINOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*TESTIS	(49)	(50)	(46)
INTERSTITIAL-CELL TUMOR	1 (2%)		
NERVOUS SYSTEM			
*BRAIN	(48)	(50)	(46)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3		6
MORIBUND SACRIFICE		1	3
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	42	49	35
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	23	18
TOTAL PRIMARY TUMORS	26	27	22
TOTAL ANIMALS WITH BENIGN TUMORS	6	10	6
TOTAL BENIGN TUMORS	6	10	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	15	13
TOTAL MALIGNANT TUMORS	20	17	16
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 2,5-DITHIOBIUREA

	CONTROL(UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	48	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	48	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(48)	(48)
FIBROSARCOMA	1 (2%)	1 (2%)	
HEMANGIOSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(47)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	4 (9%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(48)
MALIGNANT LYMPHOMA, NOS	3 (6%)	10 (21%)	6 (13%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#SPLEEN	(49)	(46)	(47)
HEMANGIOSARCOMA	1 (2%)	1 (2%)	
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#MANDIBULAR L. NODE	(40)	(33)	(39)
MALIGNANT LYMPHOMA, NOS	1 (3%)		
#MEDIASTINAL L.NODE	(40)	(33)	(39)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
#LIVER	(49)	(47)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#PEYERS PATCH	(49)	(47)	(47)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
# NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(47)	(48)
HEPATOCELLULAR CARCINOMA	2 (4%)	8 (17%)	9 (19%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(44)	(37)
ADENOMA, NOS		1 (2%)	2 (5%)
#THYROID	(41)	(45)	(45)
FOLLICULAR-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(49)	(43)	(47)
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
#OVARY	(48)	(40)	(45)
PAPILLARY ADENOCARCINOMA			1 (2%)
TUBULAR ADENOMA		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(48)	(48)
ADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	6	2
MORIBUND SACRIFICE	2	2	2
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	40	42	40
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	24	20
TOTAL PRIMARY TUMORS	15	30	30
TOTAL ANIMALS WITH BENIGN TUMORS	1	7	8
TOTAL BENIGN TUMORS	1	7	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	21	18
TOTAL MALIGNANT TUMORS	14	23	22
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2,5-DITHIOBIUREA

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 2,5-DITHIOBIUREA

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(48)
EPIDERMAL INCLUSION CYST			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
ALOPECIA		1 (2%)	
HYPERKERATOSIS			1 (2%)
ACANTHOSIS			1 (2%)
*SUBCUT TISSUE	(50)	(49)	(48)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(48)
CONGESTION, CHRONIC PASSIVE	1 (2%)		
INFLAMMATION, INTERSTITIAL	4 (8%)		
FIBROSIS, DIFFUSE	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		1 (2%)
#LUNG/ALVEOLI	(49)	(49)	(48)
HEMORRHAGE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(44)	(47)
MYELOFIBROSIS		1 (2%)	1 (2%)
HYPERPLASIA, HEMATOPOIETIC		5 (11%)	6 (13%)
#SPLEEN	(50)	(49)	(47)
FIBROSIS	1 (2%)		
SCAR			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
NECROSIS, NOS		1 (2%)	
CALCIFICATION, NOS			1 (2%)
HEMOSIDEROSIS	2 (4%)		
ERYTHROPOIESIS		1 (2%)	
*MANDIBULAR L. NODE	(49)	(46)	(44)
NECROSIS, NOS		1 (2%)	
HYPERPLASIA, PLASMA CELL	1 (2%)		1 (2%)
*MESENTERIC L. NODE	(49)	(46)	(44)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, PLASMA CELL	1 (2%)		
ERYTHROPOIESIS		1 (2%)	
*RENAL LYMPH NODE	(49)	(46)	(44)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
*HEART/ATRIUM	(48)	(48)	(47)
THROMBOSIS, NOS			1 (2%)
*MYOCARDIUM	(48)	(48)	(47)
INFLAMMATION, INTERSTITIAL	1 (2%)		
FIBROSIS	2 (4%)		
DEGENERATION, NOS	1 (2%)		
*PULMONARY ARTERY	(50)	(49)	(48)
CALCIFICATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
*SALIVARY GLAND	(50)	(49)	(47)
HYPERPLASIA, INTRADUCTAL	1 (2%)		
*LIVER	(49)	(49)	(48)
BILE STASIS		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)
HEPATITIS, TOXIC		1 (2%)	
NECROSIS, FOCAL	3 (6%)		1 (2%)
METAMORPHOSIS FATTY			2 (4%)
BASOPHILIC CYTO CHANGE			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
ANGIECTASIS			1 (2%)
#LIVER/CENTRILOBULAR	(49)	(49)	(48)
CONGESTION, PASSIVE	1 (2%)		
NECROSIS, NOS			2 (4%)
*BILE DUCT	(50)	(49)	(48)
HYPERPLASIA, NOS	2 (4%)	1 (2%)	
HYPERPLASIA, DIFFUSE			1 (2%)
#PANCREAS	(47)	(48)	(45)
INFLAMMATION, NOS	2 (4%)	1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#STOMACH	(49)	(48)	(45)
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		
#GASTRIC MUCOSA	(49)	(48)	(45)
ULCER, NOS			1 (2%)
ABSCESS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
#PEYERS PATCH	(49)	(48)	(45)
HYPERPLASIA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(48)
CONGESTION, NOS	1 (2%)		
GLOMERULONEPHRITIS, NOS	4 (8%)	1 (2%)	
NEPHROSIS, NOS	35 (70%)	47 (96%)	35 (73%)
PIGMENTATION, NOS		1 (2%)	
#KIDNEY/CORTEX	(50)	(49)	(48)
CYST, NOS		1 (2%)	
#KIDNEY/PELVIS	(50)	(49)	(48)
MINERALIZATION		1 (2%)	
#URINARY BLADDER	(50)	(46)	(47)
INFLAMMATION, ACUTE HEMORRHAGIC			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(44)	(39)
CONGESTION, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
#ADRENAL CORTEX	(50)	(49)	(46)
HYPERPLASTIC NODULE		1 (2%)	
#ADRENAL MEDULLA	(50)	(49)	(46)
HYPERPLASTIC NODULE			1 (2%)
HYPERPLASIA, FOCAL		4 (8%)	
#THYROID	(37)	(41)	(37)
CYSTIC FOLLICLES	1 (3%)	1 (2%)	
HYPERPLASIA, C-CELL	2 (5%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(49)	(48)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
#PROSTATE	(48)	(45)	(44)
INFLAMMATION, NOS	3 (6%)		
INFLAMMATION, ACUTE		3 (7%)	3 (7%)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#TESTIS	(50)	(48)	(47)
MINERALIZATION		1 (2%)	
PERIVASCULITIS	1 (2%)		
CALCIFICATION, NOS	3 (6%)		
CALCIFICATION, FOCAL	1 (2%)		
ATROPHY, NOS	11 (22%)	18 (38%)	22 (47%)
HYPERPLASIA, INTERSTITIAL CELL	4 (8%)	1 (2%)	1 (2%)
#TESTIS/TUBULE	(50)	(48)	(47)
DEGENERATION, NOS		1 (2%)	1 (2%)
CALCIFICATION, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/RETINA	(50)	(49)	(48)
CATARACT		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0100	HIGH DOSE 01-0110
ATROPHY, NOS		1 (2%)	
*EYE/CRYSTALLINE LENS	(50)	(49)	(48)
CALCIFICATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(50)	(49)	(48)
FIBROSIS, DIFFUSE	1 (2%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS			3
INFLAMMATION, CHRONIC			1
INFLAMMATION, GRANULOMATOUS			1
NECROSIS, NOS			4
OMENTUM			
INFLAMMATION, CHRONIC			1
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTOLYSIS/NO NECROPSY		1	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 2,5-DITHIOBIUREA

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(49)	(50)	(49)
CONGESTION, ACUTE PASSIVE	1 (2%)		
INFLAMMATION, INTERSTITIAL		1 (2%)	
FIBROSIS, DIFFUSE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BONE MARROW	(45)	(48)	(46)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	1 (2%)
*SPLEEN	(47)	(50)	(49)
INFLAMMATION, ACUTE			1 (2%)
HEMOSIDEROSIS	3 (6%)		
ERYTHROPOIESIS		3 (6%)	1 (2%)
CIRCULATORY SYSTEM			
*APEX OF HEART	(48)	(50)	(49)
SCAR		1 (2%)	
*MYOCARDIUM	(48)	(50)	(49)
FIBROSIS	1 (2%)		
DIGESTIVE SYSTEM			
*LIVER	(48)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NECROSIS, FOCAL	1 (2%)	2 (4%)	
METAMORPHOSIS FATTY	1 (2%)		2 (4%)
BASOPHILIC CYTO CHANGE	2 (4%)		
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)		
ANGIECTASIS		2 (4%)	
#LIVER/CENTRILOBULAR	(48)	(50)	(49)
NECROSIS, NOS			2 (4%)
*BILE DUCT	(49)	(50)	(49)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
HYPERPLASIA, NOS	2 (4%)		
HYPERPLASIA, FOCAL	1 (2%)		
#PANCREAS	(48)	(49)	(46)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#STOMACH	(49)	(48)	(47)
INFLAMMATION, NOS	1 (2%)		
#GASTRIC SUBMUCOSA	(49)	(48)	(47)
EDEMA, NOS	1 (2%)		1 (2%)
#PEYERS PATCH	(49)	(50)	(46)
HYPERPLASIA, NOS	2 (4%)		
#COLON	(49)	(47)	(45)
PARASITISM	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
GLOMERULONEPHRITIS, NOS	4 (8%)		
NEPHROSIS, NOS	29 (60%)	33 (66%)	21 (43%)
#KIDNEY/CORTEX	(48)	(50)	(49)
METAMORPHOSIS FATTY	1 (2%)		
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(49)	(48)	(48)
HYPERPLASIA, NODULAR		1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
HYPERPLASIA, FOCAL		1 (2%)	
*THYROID	(45)	(46)	(32)
HYPERPLASIA, C-CELL	2 (4%)	2 (4%)	
*PARATHYROID	(27)	(35)	(18)
HYPERPLASIA, NOS		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(49)
DILATATION/DUCTS	1 (2%)		
*MAMMARY DUCT	(49)	(50)	(49)
HYPERPLASIA, CYSTIC	1 (2%)		
*UTERUS	(46)	(49)	(46)
HYDROMETRA	1 (2%)	2 (4%)	
CYST, NOS		1 (2%)	
HEMATOMA, NOS	1 (2%)		
POLYP, INFLAMMATORY	1 (2%)		
*UTERUS/ENDOMETRIUM	(46)	(49)	(46)
HYPERPLASIA, CYSTIC		3 (6%)	1 (2%)
*OVARY	(47)	(49)	(46)
INFLAMMATION, CHRONIC	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CRYSTALLINE LENS	(49)	(50)	(49)
CALCIFICATION, NOS		1 (2%)	
*LENS CAPSULE	(49)	(50)	(49)
CALCIFICATION, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0100	HIGH DOSE 02-0110
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC		1	1
NECROSIS, NOS		1	1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	2	8
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/HISTO PERF		1	3
AUTOLYSIS/NO NECROPSY			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2,5-DITHIOBIUREA

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 2,5-DITHIOBIUREA

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	47
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(47)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, NOS			1 (2%)
ACANTHOSIS			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(47)
HEMATOMA, NOS	1 (2%)		
INFLAMMATION, ACUTE FOCAL	1 (2%)		
ABSCESS, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(47)	(50)	(47)
INFLAMMATION, INTERSTITIAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(50)	(47)
THROMBOSIS, NOS		1 (2%)	
CONGESTION, NOS			1 (2%)
ATROPHY, NOS			1 (2%)
ANGIECTASIS		1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS		2 (4%)	1 (2%)
ERYTHROPOIESIS	2 (4%)		1 (2%)
#PANCREATIC L.NODE	(40)	(41)	(40)
HYPERPLASIA, LYMPHOID		1 (2%)	
#LUMBAR LYMPH NODE	(40)	(41)	(40)
HYPERPLASIA, PLASMA CELL		1 (2%)	
#MESENTERIC L. NODE	(40)	(41)	(40)
INFLAMMATION, ACUTE			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
HYPERPLASIA, PLASMA CELL		1 (2%)	
*RENAL LYMPH NODE	(40)	(41)	(40)
HYPERPLASIA, NOS	2 (5%)		
HYPERPLASIA, PLASMA CELL		1 (2%)	
CIRCULATORY SYSTEM			
*HEART	(49)	(50)	(46)
PERIVASCULITIS		1 (2%)	
*MYOCARDIUM	(49)	(50)	(46)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
ANGIECTASIS			1 (2%)
*HEPATIC SINUSOID	(49)	(50)	(47)
LEUKOCYTOSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
*LIVER	(49)	(50)	(47)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	1 (2%)	1 (2%)	
MEGALOCYTOSIS		1 (2%)	1 (2%)
HYPERPLASTIC NODULE		2 (4%)	1 (2%)
ANGIECTASIS	1 (2%)		
*LIVER/KUPFFER CELL	(49)	(50)	(47)
HYPERPLASIA, NOS	1 (2%)		
*PANCREAS	(46)	(44)	(45)
CYSTIC DUCTS	1 (2%)	2 (5%)	
PERIVASCULITIS	1 (2%)		
NECROSIS, FAT	1 (2%)		
*STOMACH	(49)	(50)	(47)
CYST, NOS			1 (2%)
ABSCESS, NOS			1 (2%)
ACANTHOSIS			1 (2%)
*GASTRIC MUCOSA	(49)	(50)	(47)
INFLAMMATION, ACUTE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
#PEYERS PATCH	(49)	(50)	(46)
INFLAMMATION, ACUTE	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(47)
HYDRONEPHROSIS	2 (4%)		
INFLAMMATION, CHRONIC	1 (2%)		
GLOMERULONEPHRITIS, CHRONIC		1 (2%)	
GLOMERULOSCLEROSIS, NOS		3 (6%)	
HEMOSIDEROSIS			1 (2%)
#KIDNEY/GLOMERULUS	(49)	(50)	(47)
AMYLOIDOSIS			1 (2%)
#U. BLADDER/MUCOSA	(49)	(50)	(46)
INFLAMMATION, ACUTE		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(42)	(47)	(40)
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(47)
DILATATION, NOS	1 (2%)		
DILATATION/DUCTS			1 (2%)
*SEMINAL VESICLE	(50)	(50)	(47)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
#TESTIS	(49)	(50)	(46)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
#TESTIS/TUBULE	(49)	(50)	(46)
DEGENERATION, NOS		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
NERVOUS SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0120	HIGH DOSE 05-0130
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(47)
PARASITISM		1 (2%)	
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(47)
ADHESION, NOS	1 (2%)		
*MESENTERY	(50)	(50)	(47)
STEATITIS	1 (2%)		
ABSCESS, NOS	1 (2%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS	1		
NECROSIS, FAT	2		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	17	18	21
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1		
AUTO/NECROPSY/NO HISTO	1		
AUTOLYSIS/NO NECROPSY			2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 2,5-DITHIOBIUREA

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	48	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	48	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(48)	(48)
HEMORRHAGE		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(50)	(47)	(48)
INFLAMMATION, NOS		1 (2%)	
#LUNG	(50)	(47)	(48)
PERIVASCULITIS		2 (4%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(46)	(47)
ACCESSORY STRUCTURE			1 (2%)
HYPERPLASIA, PLASMA CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)
ERYTHROPOIESIS	1 (2%)		1 (2%)
#MANDIBULAR L. NODE	(40)	(33)	(39)
HYPERPLASIA, PLASMA CELL	1 (3%)		
#MEDIASTINAL L.NODE	(40)	(33)	(39)
HYPERPLASIA, NOS	1 (3%)		
HYPERPLASIA, PLASMA CELL		1 (3%)	
#LUMBAR LYMPH NODE	(40)	(33)	(39)
HYPERPLASIA, NOS	1 (3%)		
#MESENTERIC L. NODE	(40)	(33)	(39)
HYPERPLASIA, LYMPHOID			1 (3%)
#RENAL LYMPH NODE	(40)	(33)	(39)
HYPERPLASIA, NOS	1 (3%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
HYPERPLASIA, PLASMA CELL	1 (3%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(46)	(48)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, ACUTE DIFFUSE	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(47)	(48)
NECROSIS, NOS	1 (2%)		
NECROSIS, FOCAL	1 (2%)		
INFARCT, NOS	1 (2%)		
HYPERPLASTIC NODULE		3 (6%)	2 (4%)
HYPERPLASIA, FOCAL			1 (2%)
#LIVER/CENTRILOBULAR	(49)	(47)	(48)
NECROSIS, DIFFUSE			1 (2%)
*BILE DUCT	(50)	(48)	(48)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	2 (4%)		
#PANCREAS	(47)	(45)	(46)
CYSTIC DUCTS		1 (2%)	
*STOMACH	(49)	(45)	(47)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS			1 (2%)
CALCIFICATION, NOS			1 (2%)
#GASTRIC MUCOSA	(49)	(45)	(47)
INFLAMMATION, ACUTE		1 (2%)	
#PEYERS PATCH	(49)	(47)	(47)
HYPERPLASIA, LYMPHOID	1 (2%)		
#COLON	(50)	(46)	(45)
NEMATODIASIS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(47)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
INFLAMMATION, INTERSTITIAL			1 (2%)
INFLAMMATION, CHRONIC	2 (4%)	1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
PERIVASCULITIS		1 (2%)	
GLOMERULOSCLEROSIS, NOS	1 (2%)	1 (2%)	
AMYLOIDOSIS		1 (2%)	
#KIDNEY/MEDULLA	(49)	(47)	(48)
AMYLOIDOSIS			1 (2%)
#URINARY BLADDER	(50)	(45)	(47)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#U. BLADDER/MUCOSA	(50)	(45)	(47)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#U.BLADDER/SUBMUCOSA	(50)	(45)	(47)
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	16 (32%)		
PERIVASCULITIS	1 (2%)		
#U.BLADDER/MUSCULARIS	(50)	(45)	(47)
CALCIUM DEPOSIT	1 (2%)		
ENDOCRINE SYSTEM			
#THYROID	(41)	(45)	(45)
HYPERPLASIA, C-CELL	2 (5%)		
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(49)	(43)	(47)
HYDROMETRA	5 (10%)	3 (7%)	7 (15%)
NECROSIS, FAT	1 (2%)		
CALCIFICATION, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(49)	(43)	(47)
INFLAMMATION, SUPPURATIVE	2 (4%)	4 (9%)	2 (4%)
HYPERPLASIA, CYSTIC	32 (65%)	22 (51%)	28 (60%)
METAPLASIA, SQUAMOUS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
#OVARY/OVIDUCT	(49)	(43)	(47)
HYPERPLASIA, PAPILLARY			1 (2%)
#OVARY	(48)	(40)	(45)
CYST, NOS	6 (13%)	2 (5%)	4 (9%)
HEMORRHAGIC CYST		1 (3%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(50)	(48)	(48)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
*PERICARDIUM	(50)	(48)	(48)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
*MESENTERY	(50)	(48)	(48)
ABSCESS, NOS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(48)	(48)
AMYLOIDOSIS	1 (2%)	1 (2%)	
OMENTUM			
PERIVASCULITIS	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0120	HIGH DOSE 06-0130
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	5	4
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	2		1
AUTOLYSIS/NO NECROPSY		2	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 2,5-Dithiobiurea* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2,5-Dithiobiurea for carcinogenicity.

The primary reviewer for the report on the bioassay of 2,5-Dithiobiurea said that, under the conditions of test, the compound was not demonstrated to be carcinogenic in treated rats or mice. He pointed out that different batches of the compound with different purities were used. He said the shortcoming was not significant since the study was negative. Although the dosages administered to mice appeared to be adequate, the primary reviewer indicated that those used for rats appeared to have been set "arbitrarily." He said that the bioassay was probably still valid since the doses used for the rats were sufficiently high, the study was conducted for an adequate time, and the survival was satisfactory.

The secondary reviewer of the bioassay of 2,5-Dithiobiurea noted the following experimental shortcomings: 1) the stability of the compound in the diet was not determined, 2) the control group of mice was initiated five weeks after the start of the treatment groups, and 3) the examination of the thyroids should have been given special attention because of the relationship of the compound to thiourea. Despite the shortcomings, he agreed with the conclusions in the report and added that the results "give some assurance of safety" of 2,5-Dithiobiurea for humans.

There was no objection to a recommendation that the report on the bioassay of 2,5-Dithiobiurea be accepted as written.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
(David Clayson, Eppley Institute for Cancer Research, submitted a written review)
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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